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A Novel Electrochemical Sensor Based on a Mixed Diazonium/PEDOT Surface Functionalization for the Simultaneous Assay of Ascorbic and Uric Acids. Towards an Improvement in Amperometric Response Stability

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Abstract: A new electrochemical sensor was developed for the simultaneous assay of 2 major antioxidants, namely ascorbic (AA) and uric (UA) acids in neutral media. The electrode was modified by means of electropolymerized conductive poly(3,4-ethylenedioxythiophene) (PEDOT) organic films. The stability of the resulting interface was improved thanks to a thiophene-containing diazonium layer previously grafted onto the glassy carbon substrate. The morphology and electrochemical properties of all PEDOT and diazonium/PEDOT layers were characterized by Field Emission Gun Scanning Electron Microscopy (FEG-SEM) and cyclic voltammetry (CV). The best analytical performances were obtained for both biomarkers with diazonium electrodeposited under galvanostatic mode and PEDOT generated by CV. Using CV the sensitivities and detection limits were found to be 0.345 $\mu A\,cm^{-2}\,\mu M^{-1}$ and 6 μM for AA and 0.665 $\mu A\,cm^{-2}\,\mu M^{-1}$ and 1.5 μM for UA. The calibration curves were linear in the concentration range 12–1400 μM and 10–1000 μM for AA and UA, respectively. A high selectivity was observed with a detection potential difference more than 250 mV. The sensor exhibited a particularly long lifetime as well in storage (at least 85 % of the initial response recorded after one month) as in operational conditions (more than 80 % recovery after 40 successive measurements).

Keywords: Electrochemical sensors · PEDOT film · Diazonium salt · Antioxidants · Sensor stability

1 Introduction

Oxidative stress is probably one of the most studied biochemical processes for many years [1–3]. It is the consequence of an unbalance between the production of highly reactive oxygen and nitrogen species such as superoxide anion, oxygen peroxide, hydroxyl radical or nitric oxide, and the antioxidant defense system mainly based on enzymes and low molecular weight compounds [4]. Oxidative stress leads to the oxidation of lipids, proteins and nucleic acids [5–7] and is suspected to be involved in the early stages of many pathologies such as cataract, cancer, cardiovascular and neurodegenerative diseases [8,9]. Among the low molecular weight antioxidative species, ascorbic (AA) and uric (UA) acids are of particular interest since they are present in many biological fluids such as plasma, serum, urine or tears. AA is considered to be one of the most powerful hydrophilic antioxidant and plays a key role in the protection of human cells against oxidative stress. At a clinical level, it has been used for the treatment and prevention of several pathologies such as scurvy [10], common cold [11], cancers [12] or AIDS [13]. On the contrary, the role of UA with respect to the prevention against oxidative injuries is not entirely clear. On the one hand, it is commonly considered as an indicator of gout [14] but on the other hand several epidemiological studies suggest that high UA levels in serums represent a risk factor for cardiovascular diseases [15] and kidney stones [16]. Thus, both AA and UA are useful in the evaluation of oxidative stress and may be regarded as biochemical markers in a lot of pathologies. In this context, their selective and sensitive determination is of major interest for biological research and routine analysis. Numerous analytical methods have been used for the quantitative determination of AA and UA such as enzymatic methods [17], spectrofluorometry [18], HPLC analysis [19] or capillary electrophoresis [20]. The drawbacks of all these methods are mainly related to their cost and their complex experimental protocols which usually require sample pretreatment and thus a consequent preparation time. In the last two decades, electrochemical methods have been envisaged as an interesting alternative because of their specific advantages: simplicity, low cost, fast analysis and good selectivity [21,22]. However, a major problem arises due to AA and UA slow electrochemical kinetics which induce the need for high overpotentials on most common electrode materials (glassy

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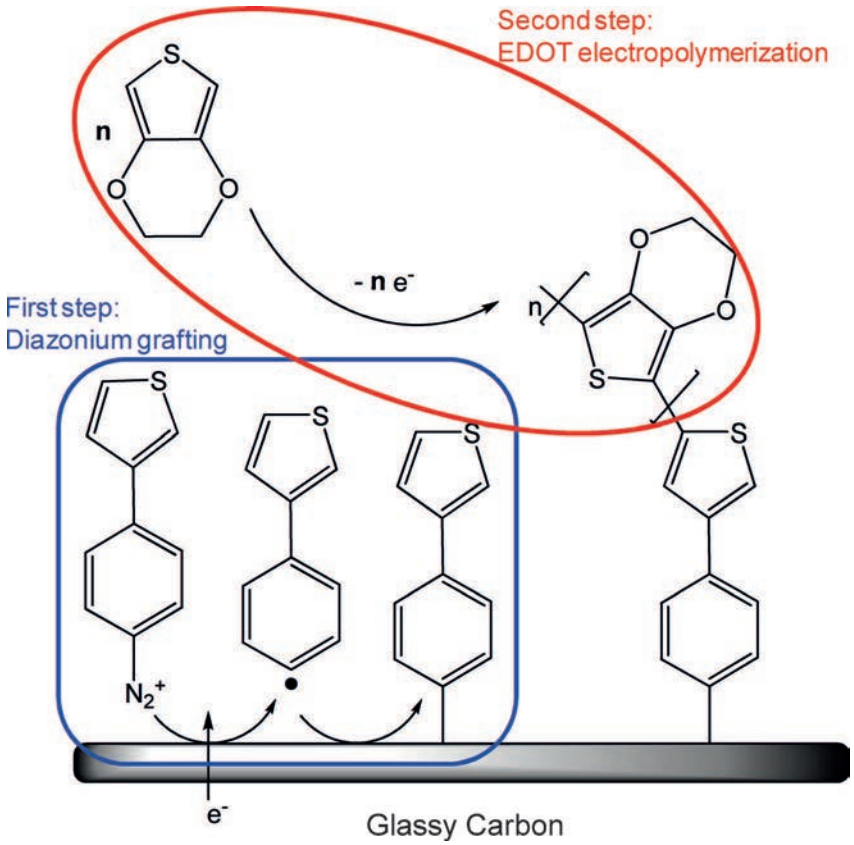

Scheme 1. Two-step functionalization principle.

carbon, gold, platinum…) [23–25]. Furthermore, both acids are oxidized at very close potentials, thus making their simultaneous assay difficult [26], especially while considering that AA and UA consistently coexist in biological fluids.

To overcome this lack of selectivity, a wide range of electrode modifications has been developed: oxidized metal electrodes [27], metal complexes [28] or electrodeposition [29], diazonium salt grafting [30], mesoporous silica [31], carbon nanotubes [26,32] or conductive polymers [21,33,34]. Among these latters, a particular attention has been paid on poly(3,4-ethylenedioxythiophene) (PEDOT) [35–37], which can be electropolymerized on most electrode materials and shows a quite high conductivity in its oxidized state and a biocompatibility in biological media.

Recently, we reported an electroanalytical process based on a gold microelectrode modified by PEDOT for the selective and quantitative determination of AA and UA in aqueous standard solution [25] and in human blood serum [38], and we also envisaged the opportunity to develop integrated microcells for the detection of both antioxidant species [39].

However, to the best of our knowledge, no stability study of the PEDOT-modified interface has been performed until yet. In a more general way and whatever the modification used, very few works have been reported which deal with the stability of the functionalized interface, although this parameter is of dramatic interest with respect to sensor reliability.

Recently, Blacha et al. have studied the polymerization of EDOT on glassy carbon (GC) electrodes modified by thiophenylbenzenediazonium (TBD) [40]. In this work, the thiophene units grafted onto the GC surface have been used to initiate the electropolymerization process and the resulting polymer layers have been found to be more ordered and more uniformly structured than the classical films produced on bare electrodes. Unfortunately, no analytical application has been proposed nor any study on the interface stability. To the best of our knowledge, this is the only report dealing with such a combined modification.

In this work, we report the elaboration of a new sensor for the simultaneous assay of AA and UA based on a GC electrode functionalized by a mixed TBD/PEDOT organic layer (Scheme 1). This latter was chosen in order to afford a covalent binding with the electrode surface to PEDOT while keeping its specific analytical properties towards AA and UA. The mixed interface was first optimized with respect to AA and UA detection by varying the electrochemical preparation mode of both TBD and PEDOT. The resulting functionalized electrodes were characterized by Field Emission Gun Scanning Electron Microscopy (FEG-SEM) and cyclic voltammetry (CV). The optimized TBD/PEDOT modified electrode was then used for the simultaneous assay of AA and UA and

its stability was examined as well in operational as in storage conditions.

2 Experimental

2.1 Chemicals and Apparatus

All products were used as received. 3,4-ethylenedioxythiophene (EDOT) ($C_6H_6O_2S$), sodium nitrite ($NaNO_2$), ascorbic acid (AA) ($C_6H_8O_6$, 99% powder) and uric acid (UA) ($C_5H_4N_4O_3$, 99% powder) were purchased from Sigma. Tetrabutylammonium perchlorate (Bu_4NClO_4) ($C_{16}H_{36}ClNO_4$) and 4-thio(phen-2-yl)aniline (TPA) ($C_{10}H_9NS$) were obtained from Sigma-Aldrich. Potassium dihydrogenophosphate (KH_2PO_4), di-potassium hydrogenophosphate (K_2PHO_4) and acetonitrile (ACN) (extra dry) were supplied by Acros Organics.

Acid solutions were prepared by dilution of 37% hydrochloric acid (HCl) from VWR using Milli-Q water (18 MΩ cm).

All electrochemical experiments were performed at room temperature using a Metrohm μ-Autolab II potentiostat interfaced to a personal computer and controlled with NOVA 1.10 software package. A classical three-electrode glass cell was used with a Metrohm platinum rod and a Radiometer saturated calomel electrode (SCE) connected to the cell by a capillary as counter and reference electrodes, respectively. All the potentials are given with respect to SCE. Working electrode was a 3 mm diameter ($A = 7.069\ mm^2$) glassy carbon (GC) rotating disk electrode from Radiometer or GC plates from OrigaLys ElectroChem SAS ($d = 5.5$ mm, $A = 23.758\ mm^2$).

When necessary, the solutions were deaerated by bubbling Nitrogen for 10 minutes. A gas stream was then maintained over the solutions during the corresponding experiments.

2.2 Electrode Preparation and Modification

Prior to modification, GC electrodes and GC plates were manually polished to a mirror-like finish successively with 9 μm, 3 μm and 1 μm diamond powder diamond suspension (Presi) on a cloth polishing pad during 2 min for each size. Between each polishing step, the surfaces were cleaned with Milli-Q water. Finally, all electrodes were rinsed in an ultrasonic 96% ethanol bath (three times for 10 minutes) and cleaned with Milli-Q water. After drying, the quality of the polishing step was verified by checking the surface state using a Nikon Eclipse LV150 optical microscope.

4-thiophenylbenzene diazonium (TBD) was prepared according to the following procedure. A cold solution of $NaNO_2$ (0.94 mg, 13.9 mmol) in Milli-Q water (1 mL) was slowly added to a 4 mL ice-cold solution of 0.125 M HCl containing TPA (2.19 mg, 12.4 mmol). The mixture was left to react at 4 °C for 1 hour and then transferred into the electrochemical cell.

Diazonium grafting onto GC was performed by dipping a freshly polished electrode in a 0.1 M HCl solution containing 2.5 mM TBD. Grafting was achieved either by cyclic voltammetry (CV) from 0.8 V to −0.2 V at $100\ mV\,s^{-1}$, by constant potential electrolysis (CPE) at −0.1 V or by constant current electrolysis (CCE) at $-0.01\ mA\,cm^{-2}$ with various amounts of charge. The electrochemical parameters were chosen in order to induce short-duration electrolyses (from 5 to 10 s) and the formation of thin organic layers onto GC whatever the method used, in accordance with previous work from our group [41].

EDOT polymerization was performed with a freshly polished electrode (or freshly TBD-modified electrode) from a 0.1 M Bu_4NClO_4 ACN solution containing 2.5 mM EDOT either by CV from 0.8 V to 1.5 V at $250\ mV\,s^{-1}$, by CPE at 1.3 V or by CCE at $0.25\ mA\,cm^{-2}$ with various amounts of charge. All these parameters were set in order to keep electropolymerization duration around 30 to 40 s and were selected with respect to previous work in similar conditions [25].

After each modification step, the electrodes were carefully rinsed with Milli-Q water before and after sonication in ethanol during 5 minutes to remove weakly adsorbed moieties.

2.3 Modified Electrode Characterization

The PEDOT and mixed TBD/PEDOT layers were systematically characterized using Field Emission Gun Scanning Electron Microscopy (FEG-SEM) and CV.

FEG-SEM measurements were performed with Quanta 250 FEG FEI equipment with an accelerating voltage of 30 kV and a working distance between 3 and 8 mm depending on the sample. In this case the working electrodes were GC plates.

Electrochemical characterizations were performed by recording cyclic voltammograms (CVs) between −0.2 V and 0.6 V (or 0.8 V for bare GC electrode) at a scan rate of $100\ mV\,s^{-1}$ in 0.1 M phosphate buffer solution (PBS) (pH 7) containing a 200 μM equimolar AA/UA mixture.

2.4 Simultaneous Assay of AA and AU

After modification, GC electrodes were immersed in 20 mL of deaerated 0.1 M PBS (pH 7) containing known amounts of AA and UA. CVs were then plotted between −0.2 V and 0.6 V at a scan rate of $100\ mV\,s^{-1}$.

3 Results and Discussion

3.1 Optimization of the Mixed Diazonium/Polymer Surface Functionalization

Prior to the design and optimization of the mixed diazonium/polymer surface functionalization, each modification was studied separately. In each case, the effect of the electrodeposition mode, namely cyclic voltammetry (CV),

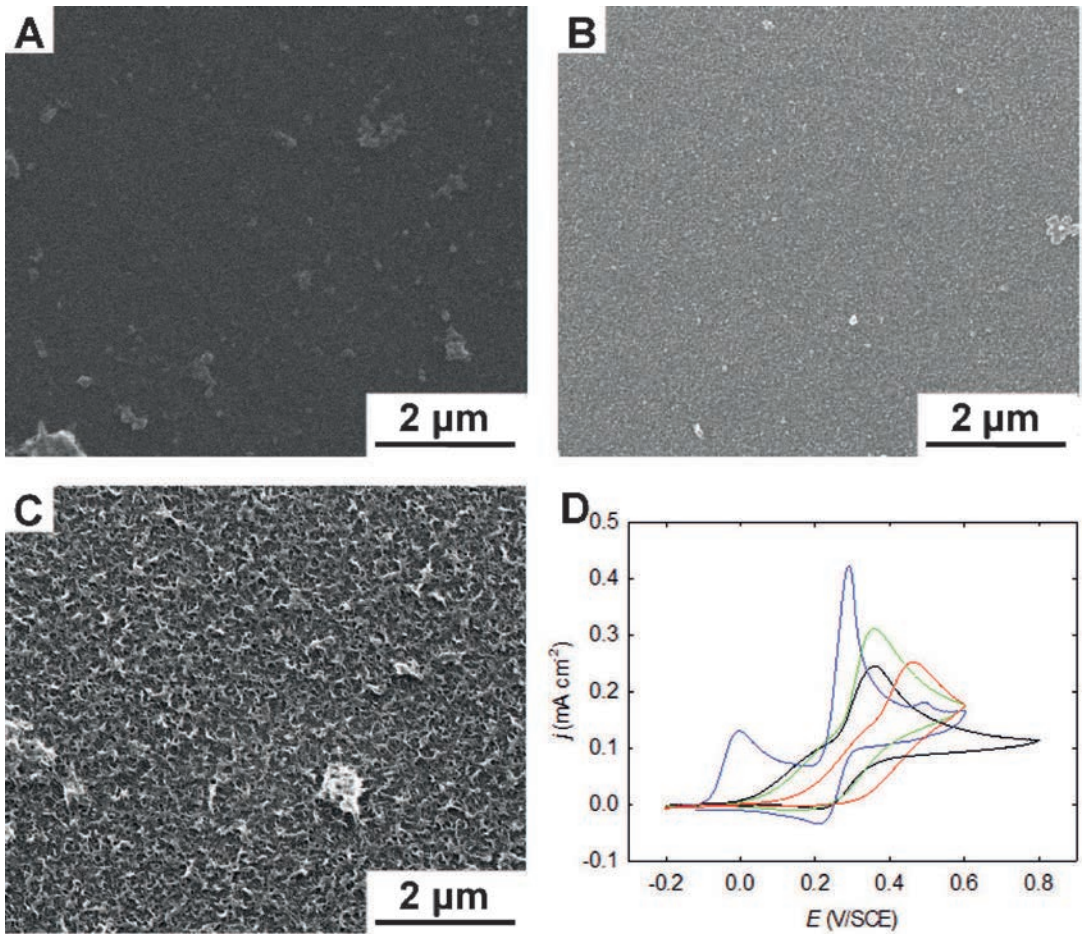

Fig. 1. (A), (B) and (C): FEG-SEM micrographs of GC electrode modified by PEDOT electrogenerated from a 0.1 M Bu_4NClO_4 ACN solution containing 2.5 mM EDOT ($q = 10\,mC\,cm^{-2}$) by: (A) CPE at 1.3 V; (B) CCE at $0.25\,mA\,cm^{-2}$; (C) CV from 0.8 V to 1.5 V at $250\,mV\,s^{-1}$. (D) CVs recorded at $100\,mV\,s^{-1}$ in 0.1 M PBS (pH 7) containing a 200 µM equimolar AA/UA mixture on bare GC (black) and on GC/PEDOT electrode prepared using the conditions corresponding to micrograph: (A) (red); (B) (green); (C) (blue).

constant potential electrolysis (CPE) and constant current electrolysis (CCE), was examined. First, EDOT was electropolymerized either by CPE at 1.3 V, by CCE at $0.25\,mA\,cm^{-2}$, or by CV from 0.8 V to 1.5 V at $250\,mV\,s^{-1}$ with a constant amount of charge (ca. $10\,mC\,cm^{-2}$). This latter value was chosen in accordance with our previous works dealing with PEDOT-modified electrodes [25]. The resulting PEDOT layers were characterized using FEG-SEM and CV (Figure 1). FEG-SEM micrographs recorded for each deposition mode show very clear differences in the resulting PEDOT structures. Both CPE (Figure 1A) and CCE (Figure 1B) afforded quite regular polymer formation onto the GC surface, this latter being all covered by compact, lumpy structures. CCE allowed a very homogeneous polymer layer to be obtained, with nearly the same lump size all over the surface, in accordance with literature data [42,43]. In comparison, CPE induced a much more heterogeneous layer, with the presence of some big and even very big lump heaps, as reported by Arteaga et al. [44]. On the contrary, the electropolymerization of EDOT by CV afforded a very different polymer structure since a very fibrous and porous layer was obtained which homogeneously covered the GC surface (Figure 1C). All these data compared well with Patra et al.'s work, in which the morphology of PEDOT deposited on stainless steel has been studied with respect to the electropolymerization mode [45].

The effect of PEDOT morphology on the analytical response towards AA and UA was investigated by recording cyclic voltammograms (CVs) in a mixture of both analytes (Figure 1D). On bare GC, an oxidation peak at 0.35 V was noticed for UA, whereas AA only appeared as a shoulder around 0.19 V (black curve). A similar curve was recorded on GC functionalized with PEDOT prepared by CPE, both anodic currents being nearly the same as on bare GC (red curve). However, a potential shift to more positive values was observed which indicated a more resistive behavior of the functionalized electrode compared to the bare one and a slowered kinetics of the electrochemical reactions. This may be due to a more difficult accessibility of both antioxydants to the active sites of the polymer or to a poor conductivity of

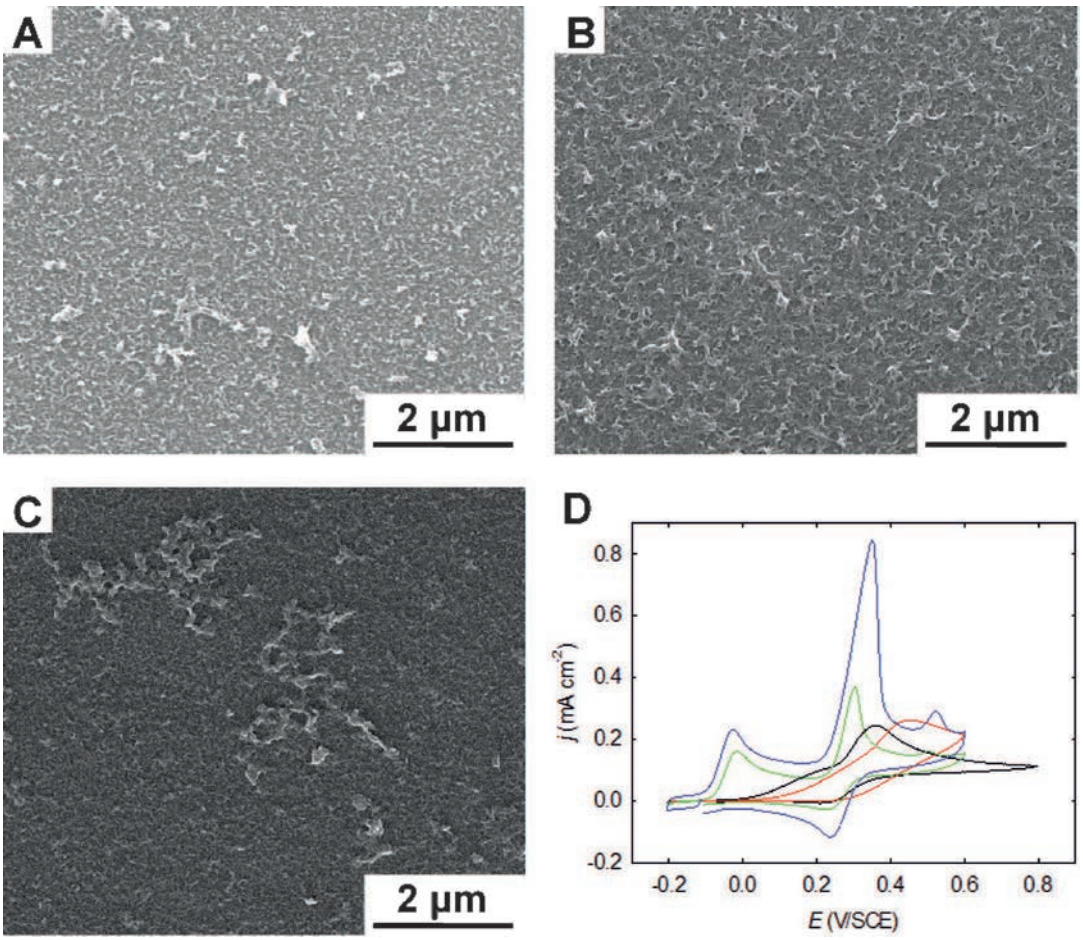

Fig. 2. (A), (B) and (C): FEG-SEM micrographs of GC electrode successively modified by TBD electrografted in 0.1 M HCl containing 2.5 mM TBD ($q = 0.1$ mC cm^{-2}) by: (A) CPE at -0.1 V; (B) CCE at -0.01 mA cm^{-2}; (C) CV from 0.8 to -0.2 V at 100 mV s^{-1} and then by PEDOT electrogenerated in a 0.1 M Bu_4NClO_4 ACN solution containing 2.5 mM EDOT ($q = 10$ mC cm^{-2}) by CV from 0.8 V to 1.5 V at 250 mV s^{-1}. (D) CVs recorded at 100 mV s^{-1} in 0.1 M PBS (pH 7) containing a 200 µM equimolar AA/UA mixture on bare GC (black) and on GC/TBD/PEDOT electrode prepared using the conditions corresponding to micrograph: (A) (green); (B) (blue); (C) (red).

the PEDOT layer [46]. When PEDOT was deposited onto GC by CCE, the oxidation peaks of AA and UA were noticed at the same potentials as on bare GC, the former peak exhibiting the same current value (green curve). The oxidation peak corresponding to UA increased 25 % on this functionalized electrode compared to unmodified one, in accordance with UA hydrophobic affinity for PEDOT [25,47]. Finally, only the GC electrode functionalized with PEDOT prepared by CV allowed a selective response towards AA and UA to be observed (blue curve). In this case, the oxidation reactions occurred at -0.02 V and 0.29 V for AA and UA, respectively, and both peak currents were significantly enhanced compared to bare GC electrode. Thus, this study confirmed that the best electroanalytical response towards AA and UA is obtained with the most porous PEDOT film, i.e. the one deposited by CV. This is in accordance with our previous works which all used this modification procedure [25,38,39].

A similar study was performed concerning the effect of the grafting mode of 4-thiophenylbenzenediazonium (TBD). First, CVs in 0.1 M HCl solution containing 2.5 mM TBD were recorded on a bare GC electrode. The first scan exhibited a broad, single reduction peak centered around -0.17 V, which disappeared upon further scanning, in accordance with the commonly reported diazonium grafting process (not shown) [48]. This grafting curve compared favorably with that recorded for the same compound in acetonitrile by Blacha et al. [40]. Grafting experiments were then performed either by CV from 0.8 to -0.2 V at 100 mV s^{-1}, by CPE at -0.1 V or by CCE at -0.01 mA cm^{-2} with a constant amount of charge (0.1 mC cm^{-2}). The resulting modified electrodes were used to record CVs in a 200 µM equimolar AA/UA mixture (not shown). Whatever the TBD grafting mode used, no signal was recorded, in accordance with a strong blocking effect of the resulting organic layer [40].

From both previous sets of experiments, it was decided to examine the mixed diazonium/polymer modification

Table 1. Influence of the amount of charge consumed for TBD grafting and PEDOT electropolymerization on peak potentials and anodic current densities (relative values) for AA and UA on GC/TBD/PEDOT modified electrode. Data were extracted from CVs recorded at 100 $mV s^{-1}$ in 0.1 M PBS containing a 200 μM equimolar AA/UA mixture. TBD was electrografted in 0.1 M HCl containing 2.5 mM TBD by CCE at $-0.01\ mA\,cm^{-2}$. PEDOT was electrogenerated in a 0.1 M Bu_4NClO_4 ACN solution containing 2.5 mM EDOT by CV from 0.8 V to 1.5 V at 250 $mV s^{-1}$.

	q (mC cm^2)	AA		UA		ΔE_p (mV)
		E_p (mV/SCE)	j/j_{max}	E_p (mV/SCE)	j/j_{max}	
TBD	0.01	−37	0.77	264	0.74	301
	0.05	−25	0.85	298	0.86	323
	0.1	−45	1	276	1	321
	0.5	−20	0.84	316	0.87	336
	1	−3	0.66	263	0.62	266
PEDOT	2.5	3	0.39	354	0.41	351
	5	7	0.91	339	0.56	332
	7.5	−9	0.87	340	0.83	349
	10	−23	1	334	1	357
	15	−7	0.79	381	0.93	388

using PEDOT prepared by CV and varying only the diazonium grafting mode. Thus TBD was grafted onto GC by CV, CPE and CCE using the conditions previously described, and EDOT was electropolymerized onto the resulting modified electrode by CV from 0.8 V to 1.5 V at 250 $mV s^{-1}$ (amount of charge consumed: 10 $mC cm^{-2}$). The SEG-FEM characterization of each deposit and the electrochemical response of the various GC/TBD/PEDOT electrodes towards AA and UA are summarized in Figure 2. Clearly, the diazonium grafting mode has a strong influence on the global morphology of the mixed organic/polymer layer formed onto GC. PEDOT deposited over TBD grafted by CV resulted in a compact layer which covered the whole surface and exhibited big lumpy substructures (Figure 2C). When grafting TBD by CPE, the resulting layer was much more porous but still exhibited some big, compact heaps (Figure 2A). Finally, when TBD was grafted by CCE the mixed layer was very porous and homogeneously structured all over the electrode surface (Figure 2B), providing a mixed layer morphology very close to that obtained while depositing PEDOT directly on bare GC. CVs recorded with each functionalized electrode in a mixture of AA and UA clearly showed that the best response was obtained when TBD was grafted by CCE, both in terms of potential separation and peak currents (Figure 2D, blue curve). The decrease of AA peak current was close to that expected according to Cottrel's law (proportional to $t^{-1/2}$) for a diffusion-controlled process. On the contrary, UA peak current exhibited a sharp decrease related to a process driven by adsorption. This is consistent with previous studies which showed that the peak current evolved linearly with the square root of scan rate for AA and with scan rate for UA [49]. TBD grafted by CV (Figure 2D, red curve) afforded nearly the same analytical response than bare GC (Figure 2D, black curve), although a little bit more resistive. Finally, the signal recorded when TBD was grafted by CPE (Figure 2D, green curve) was very close to that recorded with PEDOT alone (Figure 1, blue curve). The best electroanalytical response towards AA and UA was thus obtained when the mixed diazonium/polymer modification was performed by grafting TBD using CCE and preparing PEDOT by CV. At first glance it is surprising that a current corresponding to AA and UA oxidation was actually recorded with PEDOT/TBD/GC electrode while no signal was observed on TBD. In the latter case no redox reaction between the diazonium and the antioxidant species was expected and the barrier effect did not allow the direct oxidation of AA or UA at the GC surface. In the former case a redox reaction between species in solution and the electroactive polymer took place and then the electron transfer between PEDOT and GC surface was assumed to occur through the conjugated π bonds of the polymer skeleton (see Scheme 1) or by electron hoping. Anyway the actual electron transfer mechanism occurring on PEDOT/TBD interface is still the object of extensive discussion in the literature.

The influence of the amount of charge consumed for TBD grafting and PEDOT formation on the analytical response towards AA and UA was then explored. The corresponding modified electrodes were examined with respect to sensitivity (peak currents) for both acids and selectivity (peak separation). All the results are detailed in Table 1. The amount of charge consumed during TBD grafting (q_{TBD}) was first varied, maintaining the amount of charge consumed for PEDOT formation (q_{PEDOT}) at 10 $mC cm^{-2}$. The q_{TBD} range values corresponded to low charge values, in order to get thin diazonium layers which would not exhibit a too strong blocking effect. Except for $q_{TBD} = 1\ mC cm^{-2}$, varying q_{TBD} did not have any significant influence on the peak separation, as can be seen from the ΔE_p values. Thus, the peak currents were considered as the key feature in this case, and the best grafting charge was found to be $q_{TBD} = 0.1\ mC cm^{-2}$. This latter value was thus chosen and maintained constant while varying q_{PEDOT}. As previously observed when varying q_{TBD}, ΔE_p remained nearly constant and increased 30 mV

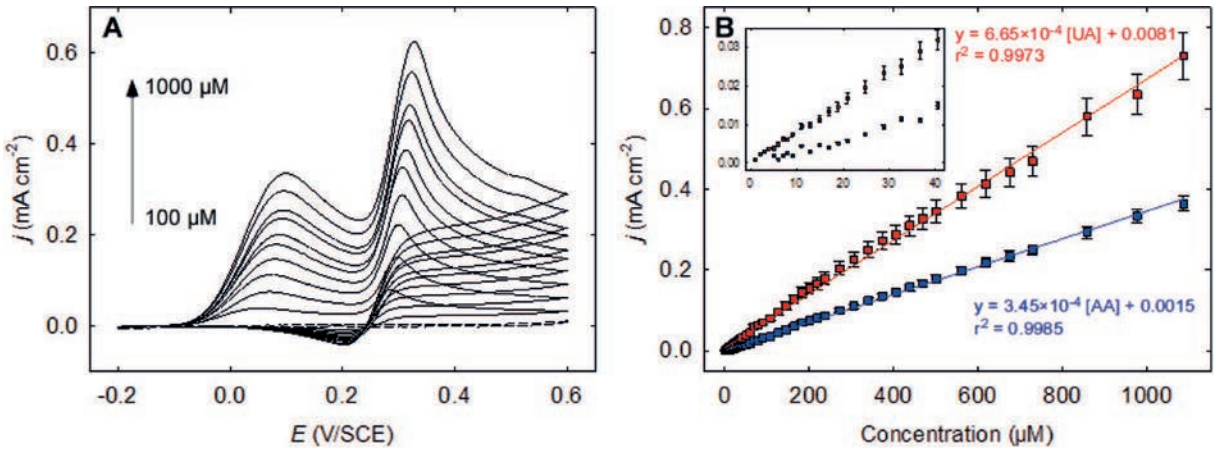

Fig. 3. (A) CVs recorded at 100 mV s^{-1} in 0.1 M PBS containing equimolar AA/UA mixtures (from 100 to 1000 µmol L^{-1}) on a GC electrode successively modified by TBD electrografted in 0.1 M HCl containing 2.5 mM TBD by CCE at −0.01 mA cm^{-2} (q = 0.1 mC cm^{-2}) and then by PEDOT electrogenerated in a 0.1 M Bu_4NClO_4 ACN solution containing 2.5 mM EDOT by CV from 0.8 V to 1.5 V at 250 mV s^{-1} (q = 10 mC cm^{-2}). (B) Calibration curve obtained for AA (blue) and UA (red).

Table 2. Analytical performances of GC/PEDOT and GC/TBD/PEDOT modified electrodes towards AA and UA simultaneous assay. Both electrodes were prepared using optimal conditions (see Section 3.1).

Electrode modification		Limit of detection (µM)	Linearity range (µM)	Sensitivity (µA cm^{-2} µM^{-1})	Repeatability (%)	Reproducibility (%)
PEDOT	AA	8 ± 1	15–1200	0.299 ± 0.027	13	6
	UA	1 ± 0.5	8–1000	0.592 ± 0.044	14	3
TBD/PEDOT	AA	6 ± 1	12–1400	0.345 ± 0.022	5	4
	UA	1.5 ± 0.5	10–1000	0.665 ± 0.053	8	7

only for the highest q_{PEDOT} value (ca. 15 mC cm^{-2}). UA peak current increased gradually while increasing q_{PEDOT} whereas AA peak current passed by a maximum value for q_{PEDOT} = 10 mC cm^{-2}. Thus this latter value was chosen as the best one for PEDOT formation. From all the results described above, the best conditions for the modification of the GC electrode were found to be: TBD grafting by CCE at −0.01 mA cm^{-2} (q_{TBD} = 0.1 mC cm^{-2}) and EDOT electropolymerization by CV from 0.8 V to 1.5 V at 250 mV s^{-1} (q_{PEDOT} = 10 mC cm^{-2}). These latter conditions were then used to prepare the mixed diazonium/PEDOT functionalized GC electrode for analytical purpose.

3.2 Analytical Performances Towards AA and UA Simultaneous Assay

The electrode functionalized by the optimized mixed TBD/PEDOT layer was used to simultaneously determine AA and UA in a mixture solution. It was first verified that the electrode afforded a linear response towards each acid separately (not shown). Then, both acids concentrations were varied simultaneously from 100 to 1100 µM and the corresponding successive CVs were recorded (Figure 3A). Whatever the concentrations, the oxidation peaks of AA and UA remained well separated. Both peak currents followed a linear trend while increasing gradually the concentrations of AA and UA (Figure 3B). The corresponding analytical performances are summarized in Table 2.

The linearity ranges provided by the functionalized electrode were found to be very wide, at least 2 orders of magnitude for UA and 3 for AA. Interestingly, the sensitivity with respect to AA detection did not vary in the absence or in the presence of UA (0.325 µA cm^{-2} µM^{-1} and 0.345 µA cm^{-2} µM^{-1} respectively), whereas the sensitivity for UA increased from 0.580 µA cm^{-2} µM^{-1} to 0.665 µA cm^{-2} µM^{-1} when operating in the presence of AA, in accordance with the EC' mechanism which takes place during the simultaneous assay of both acids [50]. The repeatability and reproducibility of the results were evaluated by recording 5 measurements for each concentration (repeatability) and by using 5 different electrodes functionalized in the same way (reproducibility). Both values were found to be lower than 10 %. It has to be noticed that the analytical performances of the TBD/PEDOT modified electrode were found to be slightly better than that of the PEDOT modified electrode (see Table 2 for quantitative feature). This may be due to a better ordered structure of the polymer brought by the diazonium layer, which induced an easier access to the active sites.

Finally, all these results were compared with literature data for both AA and UA assay in terms of sensitivity

Table 3. Comparison of the analytical performances of the GC/TBD/PEDOT electrode with literature data. Detection mode abbreviations: SWV: square wave voltammetry; DPV: differential pulse voltammetry; LSV: linear sweep voltammetry; CA: chronoamperometry.

Electrode material	Functionalization	Detection mode [a]	Sensitivity ($\mu A\,cm^{-2}\,\mu M^{-1}$)		Limit of detection (μM)		Reference
			AA	UA	AA	UA	
GC	Polyacetylthiophene	SWV	0.712	2.49	8	1	[51]
GC	RNA	DPV	0.283	8.43	5	0.2	[52]
GC	Single-walled carbon nanotube	LSV	–	0.078	–	0.2	[26]
GC	Aminophenol diazonium	CA	–	–	10	0.2	[30]
GC	Aspartic acid	DPV	–	–	10	0.2	[53]
GC	PEDOT	DPV	0.057	1.92	7.4	1.4	[54]
GC	PEDOT	CV	–	0.679	Excess	1	[24]
μAu [a]	PEDOT	DPV	0.875	3.08	2.5	1.5	[25]
GC	TBD/PEDOT	CV	0.345	0.665	6	1.5	This work

[a] In this case, a gold microelectrode was used.

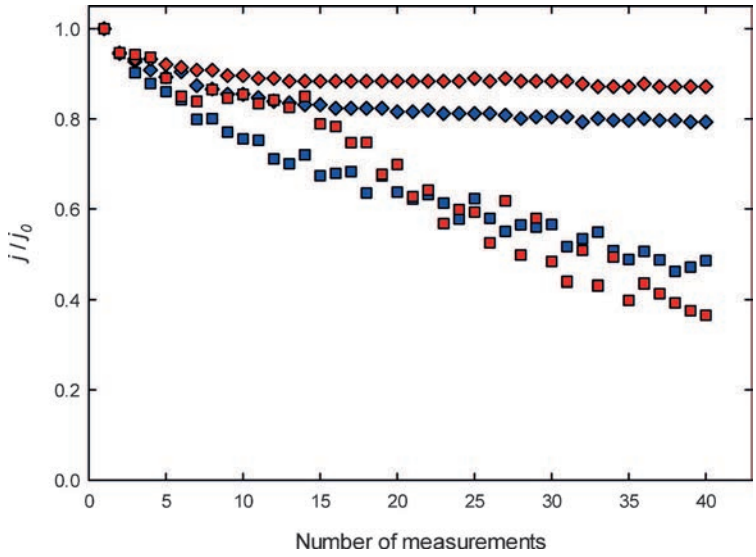

Fig. 4. Evolution of relative peak current density values j/j_0 corresponding to AA (blue) and UA (red) oxidations with consecutive CVs recorded at 100 $mV\,s^{-1}$ in 0.1 M PBS (pH 7) containing 200 μM equimolar AA/UA mixture on GC/PEDOT (■) and GC/TBD/PEDOT (◆) modified electrodes. TBD was electrografted in 0.1 M HCl containing 2.5 mM TBD by CCE at −0.01 $mA\,cm^{-2}$ (q = 0.1 $mC\,cm^{-2}$). PEDOT was electrogenerated in a 0.1 M Bu_4NClO_4 ACN solution containing 2.5 mM EDOT by CV from 0.8 V to 1.5 V at 250 $mV\,s^{-1}$ (q = 10 $mC\,cm^{-2}$).

and limit of detection (Table 3). The sensitivity reported here for AA compared well with previously reported values, whatever the electrochemical detection mode. For UA, our results were very close to those reported by Kumar et al. [24] who also used CV, but were found to be far below the ones found by using the more sensitive SWV and DPV as the detection mode. The limits of detection for both acids also compared well with literature data. It has also been verified that GC/TBD/PEDOT electrode allowed the simultaneous and selective detection of AA and UA in the presence of dopamine, the differential pulse voltammogram being similar to that previously reported by our group with PEDOT/μAu electrode [25].

3.3 Stability of the Response of the Mixed TBD/PEDOT Interface Towards AA and UA Oxidation

The stability of the amperometric response of the mixed diazonium/polymer functionalized GC electrode was examined and compared to that of the more classical PEDOT-modified GC electrode as well in operational as in storage conditions. First, Figure 4 shows the evolution of the peak currents of AA and UA for both electrodes as a function of the number of consecutive CVs. The results are provided as the relative current density j/j_0, j being the current density recorded at each measurement and j_0 being the current recorded during the first measurement. Clearly, the responses obtained on the 2 different electrodes experienced very distinct evolutions. GC functionalized using the new mixed diazonium/PEDOT layer initially exhibited a significant decrease of the peak

currents between the first and third measurement (ca. 9% and 7% for AA and UA, respectively). Then the response rapidly stabilized (from scan 10) and remained nearly constant. After 40 measurements, the mixed TBD/PEDOT functionalized GC electrode exhibited 79% and 87% recovery of the initial peak currents for AA and UA, respectively. On the contrary, the electrode modified with only PEDOT afforded a constant decrease in both currents, to reach only 48% and 36% recovery of the initial responses for AA and UA respectively, after 40 measurements. The stability of both electrodes was also evaluated under storage conditions by recording CVs before and after leaving the electrodes during one month without any particular care. The TBD/PEDOT functionalized electrode allowed 88% and 85% of the initial response to be recovered with respect to AA and UA, respectively. The PEDOT/GC electrode only allowed 60% and 85% recovery for AA and UA respectively. Thus, the presence of TBD strongly enhances the stability of the resulting functionalized electrode. This is mainly due to the covalent binding it affords with the GC surface. Moreover, due to the thiophene unit born by the diazonium phenyl group, TBD is entangled in the PEDOT structure during the electropolymerization step. The resulting mixed organic/polymer layer thus exhibits improved stability while keeping the analytical properties of PEDOT. Compared to our previous work [25] this improved stability induced by grafted TBD layer undoubtedly increases the opportunity to exploit such a modified electrode in clinical biology analysis. Works are in progress in our laboratory for the analysis of real medium such as blood serum.

4 Conclusions

In this work, we described the preparation and optimization of a GC electrode modified by a mixed diazonium/PEDOT and we determined the analytical performances of this resulting new electrochemical sensor dedicated to AA and UA simultaneous assay. The best results were obtained using a GC electrode functionalized by TBD prepared by CCE and by PEDOT polymerized using CV. The new mixed TBD/PEDOT functionalized GC electrode exhibited sensitivities, linear concentration ranges and limits of detection which compared well with literature data. The stability of the analytical response in storage and operating conditions were also examined and proved to be satisfactory. By comparison with an electrode modified by only PEDOT, it was demonstrated that this enhanced stability was brought by the diazonium layer which was grafted onto the GC surface before the electropolymerization step. The resulting mixed layer thus exhibited the stability of a diazonium and the analytical performances of PEDOT. Works are in progress to better understand the structural interactions between TBD and PEDOT and to perform analytical tests in clinical biology.

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